Title

Sex-specific differences in effect of prenatal exposure to dioxin-like compounds on neurodevelopment in Japanese children: Sapporo cohort study

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Abstract

BACKGROUND: Consistent reports are not available on the effects of dioxin-like polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins (PCDD)/ polychlorinated dibenzofurans (PCDF) (dioxin-like compounds [DLCs]) on child neurodevelopment. Further, the effect of background-level exposure to individual DLC isomers is not known.

OBJECTIVES: We carried out the Sapporo cohort study to evaluate the effect of prenatal exposure to each DLC isomer on child neurodevelopment at 6 and 18 months of age, and assessed sex-specific differences in these effects.

METHODS: The levels of all and each individual DLC isomers were estimated in maternal peripheral blood. Neurodevelopment was evaluated using the Bayley Scales of Infant Development-2nd Edition for 6-month-old infants (n = 190) and 18-month-old children (n = 121).

RESULTS: In male children, levels of 10 DLC isomers were significantly negatively associated with the Psychomotor Developmental Index (PDI) at 6 months of age after adjustment for potential confounding variables. However, at 18 months of age, these associations were absent. In female children, the level of only one DLC isomer was significantly negatively associated with PDI at 6 months of age. However, in contrast to the male children, the levels of six DLC isomers in 18-month-old female children were significantly positively associated with the Mental Developmental Index.

CONCLUSIONS: These findings indicate that adverse neurodevelopmental effects of prenatal background-level exposure to DLCs may be stronger in male children.

Keywords

dioxin-like compounds; child development; prenatal exposure; background-level; sex-specific differences

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1. Introduction

Endocrine-disrupting chemicals such as polychlorinated biphenyls (PCBs), dioxin-like PCBs and polychlorinated dibenzo-p-dioxins (PCDDs) / polychlorinated dibenzofurans (PCDFs) (dioxin-like compounds [DLCs]) have various harmful health effects. Previous studies have demonstrated their adverse effects on growth and development of children in heavily polluted areas where pregnant women had consumed oil and fish contaminated with PCB and/or PCDFs. Regarding cases of Yu-cheng (or oil disease) in Taiwan, a follow-up survey of children whose mothers had eaten cooking oil contaminated by PCB and PCDFs while pregnant was conducted to assess their growth and neurodevelopment from 2–12 years of age. The study revealed that prenatal exposure caused persistent cognitive and behavioral problems, but some improvement of these issues was observed at more advanced ages (Lai et al., 2001). In Lake Michigan, another PCB polluted area; a follow-up survey of children whose mothers had eaten PCB contaminated fish while pregnant investigated their neurodevelopment from the age of 5 months to 11 years. It was found that prenatal exposure to PCBs was significantly associated with a lower full-scale and verbal IQ score in the 11-year-old children (Jacobson and Jacobson,1996). Moreover, prenatal PCB exposure was significantly negatively associated with cognitive development only in children who had breast-fed for <6 weeks at 4 and 11 years of age, respectively (Jacobson and Jacobson, 2002). In children who had not been breast fed, prenatal exposure to PCBs was associated with greater impulsivity, poor concentration, as well as poor verbal, pictorial, and auditory working memory at 4 and 11 years of age, respectively (Jacobson and Jacobson,2003).

Since the late 1980s, several studies have reported the effects of pre- and postnatal background-level exposure to environmental chemicals such as DLCs on child neurodevelopment—the Netherlands study (Huisman et al., 1995, Koopman-Esseboom et al., 1996, Vreugdenhil et al., 2002a, Vreugdenhil et al., 2002b, Lanting et al., 1998, Patandin et al., 1999), the German Duisburg study (Wilhelm et al., 2008b, Neugebauer et al., 2015,

Nowack et al., 2015, Winneke et al., 2014), the Norwegian Mother and Child Cohort Study (MoBa) (Caspersen et al.,2016a, Caspersen et al.,2016b) and the Sapporo cohort study (Nakajima et al., 2006). In the Netherlands study, the participating children were segregated into a breast-fed and a formula-fed group, and breast milk in the breast-fed group was used to evaluate the exposure level. The total PCB/dioxin toxicity equivalency quantity (TEQ) value, indicating the dioxin concentration, in the infant and school-going subjects was reported to be approximately 63–68 ng/kg fat; another study reported lower TEQ values in the breast milk than in the maternal blood (Todaka et al., 2011). In the German Duisburg study, both maternal blood and breast milk were used for exposure evaluations, and the total PCB/dioxin TEQ value was estimated at approximately 19 pg/g lipid. In these two studies, development, intelligence, and cognitive function tests were conducted from a few weeks after birth to school-going age, and dioxin exposure was often negatively associated with neurodevelopment in infants under 8 months of age (Huisman et al., 1995, Koopman-Esseboom et al., 1996). However, this negative effect became undetectable as the children grew up (Vreugdenhil et al., 2002a, Vreugdenhil et al., 2002b, Lanting et al., 1998). Moreover, other studies have reported a lack of association between PCB/dioxin levels and neurological development at 12, 18, and 24 months of age (Koopman-Esseboom et al., 1996, Wilhelm et al., 2008b). Furthermore, some previous studies have also reported sex-specific differences in the effect of dioxins on children’s play and interpersonal behavior (Nowack et al., 2015, Winneke et al., 2014). Accordingly, increased feminine play behavior in boys and reduction of femininity in girls has been reported in one study (Winneke et al., 2014). Therefore, a consensus on the effects of prenatal exposure to dioxins on child neurodevelopment has not been established. The results of MoBa, a large cohort study that recruited pregnant women between 2002 and 2009, the concentrations of TEQ values of PCB 153 and dioxin-like PCB (dl-compounds) recorded were based on a validated food frequency questionnaire (FFQ), and was answered during the participants’ mid-pregnancy. Outcome

measures were later performed on their children for language and communication skills at the age of 3 years (Caspersen et al.,2016b), and for ADHD symptoms, intellectual function, cognitive function, and language skills and so forth at 3.5 years of age (Caspersen et al.,2016a). These studies showed that the associations between exposure to high concentrations and lower language skill scores at 3 and 3.5 years, respectively, were only significant for the girls in this cohort.

In a previous study, we examined the effects of pre- and postnatal background-level exposure to environmental chemicals such as DLCs on the psychomotor development and cognitive functions in 6-month-old children from Sapporo, Japan (n = 134) (Nakajima et al., 2006). In that study, the mean total dioxin TEQ value in maternal blood was estimated at 18.8 pg/g lipid, and was not significantly related to indices of neurodevelopment, namely the Mental Development Index (MDI) and the Psychomotor Development Index (PDI). In contrast, higher concentrations of some DLC isomers, as well as higher concentration of total DLC and the total DLC TEQ values, were significantly correlated with lower PDI scores, consistent with the Netherlands study. However, due to the small sample size and the high cost of dioxin level measurements, we were not able to explore the sex-specific developmental effects at the time (Nakajima et al., 2006). Furthermore, because the studies conducted in the Netherlands and Germany used total PCB, total polychlorinated dibenzo-p-dioxin (PCDD) / polychlorinated dibenzofuran (PCDF), and/or dioxin TEQ as estimates of exposure levels, the effect of individual DLC isoforms on neurodevelopment remains unclear.

In the present study, we performed detailed measurements of individual DLC isomers and total DLC concentration levels in maternal blood. Using the Bayley Scales of Infant Development 2nd Edition (BSID-II) to assess neurodevelopment, we examined the relationship between the levels of each DLC isomer and neurodevelopment in children aged 6 and 18 months of age. Further, we also assessed the sex-specific differences on these effects.

2. Materials and Methods

2.1. Study population

This study was planned as a prospective study as part of the Hokkaido Study on Environment and Children's Health. Five hundred and fourteen pregnant women were recruited from the Sapporo Toho Hospital in Hokkaido, Japan between July 2002 and October 2005 (Kishi et al., 2013). All subjects were Japanese natives and were residents of Sapporo and its surrounding areas. Of the 514 subjects (the Sapporo cohort) who agreed to participate in the postal questionnaire-based study, 333 (66.1% of all study subjects) agreed to participate in the follow-up study to assess their child's neurodevelopment at the time of recruitment. After the second trimester of their last pregnancy, the study subjects completed a self-administered questionnaire designed to collect information on their dietary habits, exposure to chemicals during daily life and at work site, home environment, smoking habit, and their medical histories and that of their partners. The prenatal information of the participating mothers and children was collected from their medical records. Among 333 mothers who agreed to participate in the follow-up survey of their child's neurodevelopment at recruitment, 275 children (82.5%) at 6 months of age and 204 children (74.2%) at 18 months of age completed the neurodevelopment test. All study subjects provided written informed consent and the study was approved by the institutional ethical board for epidemiologic studies at Hokkaido University Graduate School of Medicine and Hokkaido University Center for Environmental and Health Sciences.

2.2. Exposure measures

A 40-mL venous blood sample was collected from all participating mothers after the second trimester of their last pregnancy. In cases where a sample could not be collected due to gestational anemia, the sample collection was performed during post-delivery hospitalization. All samples were stored at -80 °C until analysis. The procedure of exposure measurement in

this study has been described in detail elsewhere (Todaka et al., 2011).

The maternal blood DLC concentrations were measured using high-resolution gas chromatography or high-resolution mass spectrometry (Agilent 6890, Agilent Technologies Inc., Palo Alto, CA, USA; AutoSpec-Ultima NT, Micromass Ltd., Manchester, UK, respectively) equipped with a solvent-cut large-volume injection system (SGE Ltd., Victoria, Australia) at Fukuoka Institute of Health and Environmental Sciences. The levels of each individual DLC isomers (7 PCDDs, 10 PCDFs, 4 non-ortho PCBs, and 8 mono-ortho PCBs) were measured (Nakajima et al., 2006) and their total TEQ values were calculated (Iida and Todaka, 2003, Todaka et al., 2003).

2.3. Developmental measures

We used the BSID-II (Bayley, 1993) to assess the infants' mental and psychomotor (motor) development at 6 and 18 months of age. The BSID-II was adapted and used as described previously (Nakajima et al., 2006). Because the BSID-II test items vary with the assessment age, we strictly limited the assessment period (5 months 16 days to 6 months 15 days for the 6-month and 18-month age groups, 16 days to 19 months, and 15 days for the 18-month age group) to avoid the influence of test item variations. To carry out the developmental assessment, the infants or children were brought to a community center in Sapporo where they were tested in a quiet, private room in the presence of one or both parents. Three occupational therapists with clinical experience in developmental disabilities and blinded to the children's DLC exposure level, performed the evaluations. One examiner assessed all participating children and assigned the scores, which were then double-checked through video recordings of the initial assessment by two other examiners.

We utilized the home environment questionnaire (Anme et al., 1997) to assess the environmental condition of the subjects at the time of the developmental tests.

2.4. Data analysis

In this study, the sample size was restricted because of the high cost of dioxin analysis. Therefore, we did not analyze the specific sample size before stating this analysis. However, we analyzed achievable significances posteriori, and standardized regression coefficients, which we were able to achieve statistical significances with 80% power in simple liner regressions of 6-month boys, 6-month girls, 18-month boys, and 18-month girls were 0.274, 0.285, 0.345, and 0.343, respectively. We analyzed data from participating mothers with no serious illnesses or complications during pregnancy and delivery, and singleton full-term babies (37–42 weeks' gestation) with Apgar scores of >7 at 1 min; no congenital anomalies or diseases; and who had completed BSID-II assessment within the limited assessment period.

We performed multiple regression analysis to examine the association between BSID-II scores (both MDI and PDI) and maternal blood DLC levels for all study subjects. We also performed the multiple regression analysis separately for male and female subjects to identify sex-specific association differences. Variables that demonstrated significantly different BSID-II scores in this study, as well as risk factors known or implicated in infant and/or child neurodevelopment, were included in the regression model. Furthermore, on comparing the DLC levels based on blood sampling time (during pregnancy vs. after delivery) by Mann-Whitney test, we observed significant differences in the levels of 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin (HpCDD; $p < 0.01$); octachlorodibenzodioxin (OCDD; $p < 0.01$); 1,2,3,4,7,8-hexachlorodibenzofuran (HxCDF; $p < 0.05$); 1,2,3,6,7,8-HxCDF ($p < 0.01$); 1,2,3,4,6,7,8-HxCDF ($p < 0.01$); total PCDD ($p < 0.01$); and total PCDD/PCDF ($p < 0.01$). We therefore included blood sampling time as one of the covariates in our regression model. The maternal blood DLC levels were logarithmically transformed and the regression analysis was adjusted for gestational age (days), caffeine intake during pregnancy (mg/day), economic status, smoking during pregnancy, fish intake during pregnancy (inshore and deep-sea fish), index of child care environment, and blood

sampling time. Multicollinearities were checked using variance inflation factor (VIF: 1.1–1.3), and residuals were checked using QQ plot. Results were considered significant if the p value was <0.05. All analyses were conducted using the IBM-SPSS statistics software version 20 (IBM Japan, Ltd.）.

3. Results

Based on our inclusion and exclusion criteria, 191 mother-infant pairs in the 6-month-old group and 122 mother-child pairs in the 18-month-old group were included in this study. One mother-infant pair was excluded due to extremely high levels of PCDF (1,2,3,4,6,7,8-Heptachlorodibenzofuran[HpCDF], 1,2,3,4,7,8,9-HpCDF, octachlorodibenzofuran) in the maternal blood, and subsequently, 190 pairs in the 6-month-old group and 121 pairs in the 18-month-old group were included in the final analyses.

Characteristics of the participating mothers and children in the 6- and 18-month-old group are presented in Table 1. In the 6-month-old group, the mean (± SD) maternal age was 31.1 ± 4.7 years and 12.1% of the mothers continued to smoke during pregnancy. Of the participating mothers, 43.2% consumed inshore fish and 53.2% consumed deep-sea fish at least once per week during pregnancy. Among the infants, 52.1% were first-born and 57.4% were breast-fed for 3 months. The 18-month-old group exhibited similar characteristics. The mean MDI and PDI scores were 90.6 ± 5.8 and 89.9 ± 10.7, respectively, in the 6-month-old group, and 82.2 ± 10.7 and 85.2 ± 10.9, respectively, in the 18-month-old group. These values were lower than the standardized BSID-II score of 100.

The maternal blood DLC levels (pg/g lipid) in the 18-month-old group are presented in Table 2. For the purposes of analysis, subjects with DLC levels below the detection limit were assigned values equal to half the detection limit (Longnecker et al., 2000). The median

(25^{th}–75^{th} quartiles) levels of total PCDDs/PCDFs TEQ, total co-planar PCBs TEQ, and total dioxin TEQ were 9.8 (7.4–12.5), 5.1 (3.3–7.1) and 15.1 (11.2–19.6) pg TEQ/g lipid, respectively.

To identify the sex-specific differences in the effect of DLC exposure, we stratified the mother-child pairs based on sex and conducted further statistical analyses. Among the 6-month-old male infants, those with non-working mothers ($p < 0.01$) and non-first born ($p < 0.05$) exhibited significantly lower MDI scores. Further, the gestational age (days) was significantly positively associated with both MDI ($r = 0.23$, $p < 0.05$) and PDI scores ($r = 0.27$, $p < 0.01$). In 6-month-old female infants, caffeine intake during pregnancy was significantly negatively associated with the PDI score ($r = -0.21$, $p < 0.05$) (see Table A1).

Among the 18-month-old male children, those with non-smoking mothers had significantly higher MDI scores ($p < 0.05$), and the index of child care environment was significantly positively associated with the MDI score ($r = 0.33$, $p < 0.05$). However, in the 18-month-old female children, higher MDI score was observed in the higher income group ($p < 0.05$). In addition, while mother's alcohol intake during pregnancy was significantly positively associated with the PDI score ($r = 0.26$ $p < 0.05$), the duration of breastfeeding (months) was significantly negatively associated with the PDI score ($r = -0.27$, $p < 0.05$) (see Table B1).

Table 3 shows the association between maternal blood DLC levels and the BSID-II scores for 6- and 18-month-old male children. In the 6-month-old group, only the level of PCDD isomer 1,2,3,4,6,7,8-HpCDD ($p < 0.05$) was significantly negatively associated with the MDI score. In contrast, levels of individual PCDD isomers 2,3,7,8-tetrachlorodibenzo-p-dioxin ($p < 0.05$), 1,2,3,4,6,7,8-HpCDD ($p < 0.05$), and OCDD ($p < 0.05$); PCDF isomers 2,3,7,8-tetrachlorodibenzofuran ($p < 0.05$), and 1,2,3,7,8-pentachlorodibenzofuran ($p < 0.01$); mono-ortho PCB isomers #114 ($p < 0.05$), #156 ($p < 0.05$), #157 ($p < 0.05$), #167 ($p < 0.05$), and #189 ($p < 0.05$); total levels of all DLCs ($p < 0.05$) except total PCDF and total non-ortho PCB; and the WHO-05 total levels of mono-ortho PCB TEQ ($p < 0.05$), and total coplanar

PCB TEQ ($p < 0.05$), were all significantly negatively associated with the PDI scores. In the 18-month-old group; however, no significant associations were observed.

Table 4 shows the association between the maternal blood DLC levels and the BSID-II scores for 6- and 18-month-old female children. The level of PCDD isomer 1,2,3,7,8-pentachlorodibenzo-p-dioxin ($p < 0.05$) was significantly positively associated with the 6-month MDI score, while the level of only mono-ortho PCB isomer #123 ($p < 0.05$) was significantly negatively associated with the PDI score. In contrast to the observation in the 18-month-old male children, the levels of individual PCDF isomer 1,2,3,4,6,7,8-HpCDF ($p < 0.05$); non-ortho PCB isomer #169 ($p < 0.05$); and mono-ortho PCB isomers #114 ($p < 0.05$), #156 ($p < 0.05$), #157 ($p < 0.05$), and #189 ($p < 0.05$), were all positively associated with the MDI scores for the 18-month-old female children.

4. Discussion

The results presented here reveal that for male children, while the maternal blood levels of several individual isomers and the total and TEQ levels of some DLCs were significantly negatively associated with the PDI scores at 6 months of age, this negative association disappeared by the age of 18 months. For the female children; however, the maternal blood level of only one mono-ortho PCB isomer was significantly negatively associated with the PDI score at 6 months of age, and this negative association disappeared at 18 months of age. Moreover, total level and TEQ values of DLCs in the maternal blood at both 6 and 18 months of age had no significant association with the MDI scores. However, the level of one PCDD isomer at 6 months of age, and the levels of one PCDF isomer, one non-ortho isomer, and several mono-ortho isomers, at 18 months of age were significantly positively associated with the MDI scores.

Previous studies evaluating the adverse effects associated with prenatal exposure to

environmental chemicals such as DLCs have utilized various outcome measurements. In a previous cohort study conducted by our group, we identified a significant adverse effect of exposure to DLCs (total PCDDs TEQ level, total PCDDs/PCDFs TEQ level, and total TEQ level) on birth weight only among the male infants (Konishi et al., 2009). Our observation indicated that male infants were more susceptible than female infants to prenatal DLC exposure. Previous studies have not examined the sex-specific neurodevelopmental effects of DLC exposure in children under 18 months of age. However, the New Bedford, Massachusetts (USA) birth cohort study (Sagiv et al., 2012) observed a significantly negative association between the cord serum PCB levels and neuropsychological endpoints only in 8-year-old male children, thus providing evidence for potential sex-specific adverse neurodevelopmental effects, even though direct DLC level estimations were not performed.

In the present study, we observed a positive association between the levels of several individual DLC isomers and MDI scores in the 18-month-old female children. The above-mentioned New Bedford birth cohort study (Sagiv et al., 2012) has also reported a significant positive association between higher organochlorine exposure in female children and shorter mean reaction time in the continuous performance test. This is contrary to the hypothesized negative effect of PCBs on neurodevelopment, and the authors speculated that this unexpected finding among the female children might be attributable to residual confounding factors.

The German Duisburg cohort study quantified testosterone and estradiol levels in maternal cord serum. Here, the hormonal levels decreased on PCB/dioxin exposure, and the decrease in testosterone level was more apparent in the cord serum of female infants, while the estradiol level decrease was more apparent in the cord serum of male infants. Further, this reduction in the hormone levels was more strongly associated with the dioxin level rather than the level of indicator PCBs (Cao et al., 2008). In another study examining the longitudinal association between sex-specific quartiles of bioavailable testosterone (BioT)

concentrations and the rate of developmental delay, male children in the highest BioT quartile were reported to have an increased risk of clinically significant language delay during the first 3 years of life. In contrast, higher level of BioT was shown to reduce the risk of language delay among female children (Whitehouse et al., 2012). The significant decrease in testosterone levels in female children exposed to higher dioxin concentrations, as reported in the Duisburg study, cannot be reconciled with the above-mentioned protective effect of higher BioT concentration on language delay among the female children. However, these studies emphasize the involvement of sex hormones in the sex-specific neurodevelopmental effects of background-level exposure to DLCs.

Our results on the negative association of DLC levels with the mental and motor development of 6-month-old male infants is largely consistent with our previous observations for this age group (Nakajima et al., 2006). However, these negative associations between DLC concentrations and the neurodevelopmental scores (both MDI and PDI) could not be demonstrated at 18 months of age. Thus, while the adverse neurodevelopmental effects of DLC exposure during infancy is evident at 6 months of age, these effects become increasingly difficult to detect as the child grows older. Our findings are consistent with the results of a Dutch study that examined the effect of prenatal PCB exposure on child development at 3, 7, and 18 months of age (Koopman-Esseboom et al., 1996). The authors reported a significant negative effect of prenatal PCB exposure on the PDI score at 3 months of age ($p < 0.02$); however, at 18 months of age, development was no longer affected by PCB/dioxin exposure and feeding type as breast-fed vs. formula-fed.

As stated in our previous study (Nakajima et al., 2006), the mean maternal blood total dioxin TEQ we observed was lower than that reported in another study conducted in a different region in Japan, targeting children of the same age (Watanabe, 2000). To allow for a direct comparison between exposure levels reported in various studies, the median value for maternal serum PCB 153 has been used by some researchers to provide a uniform exposure

index (Longnecker et al., 2003). In our previous study (Nakajima et al., 2006), the median PCB 153 value among the 64 study subjects was 22.9 ng/g lipid, and this contamination level was approximately one-third to one-sixth that of the values reported in the studies conducted in 11cities(U.S.), North Carolina, Düsseldorf, and the Netherlands. MoBa reported that, only the girls in their cohort showed a significant inverse association between exposure variables and language skill score at 3 and 3.5 years of age, respectively (Caspersen et al.,2016a, Caspersen et al.,2016b). In MoBa, median PCB-153 in maternal serum among subsamples was 38 ng/g lipid (Caspersen et al.,2016a) which was approximately twice that observed in our study's cohort. The Duisburg study, using different indices of exposure (PCB 138, 153, 180, and the sum of four PCB congeners [Σ]), reported that the exposure levels observed in the Düsseldorf study cohort were 2–3-times higher than their results. Thus, while the Düsseldorf study reported a significant correlation between high PCB levels and negative effect on children's neurodevelopment, the Duisburg study found no such correlation due to the low level of PCB exposure in their cohort (Wilhelm et al., 2008a). While we did not use the same biomarkers as the Duisburg study in our current work to measure exposure, our study subjects were exposed to an equivalent or lower concentration of DLCs. Since, in our study, the negative effects of dioxin exposure on neurodevelopment disappeared at 18 months of age, we similarly speculate that the lower dioxin level measured in our study subjects was not sufficient to induce negative effects on the later stages of child neurodevelopment. As mentioned above, dioxin exposure levels in developed countries have been decreasing, but it is necessary to continue to take measures to restrict the formation of dioxin.

Our study has potential limitations. We used the results of BSID-II for neurodevelopmental assessment in our cohort. The BSID-II was designed for use in the United States, and due to cultural and verbal differences between Japan and the United States, the BSID-II must be used with care. However, a previous study comparing the BSID-II and the Kyoto Developmental Test (Oka et al., 2005), reported high correlation and

reproducibility between the two, despite cultural differences (Huang et al., 2000). In addition, as the BSID-II test items vary with the assessment of age, we strictly limited the assessment period to avoid the influence of test item variations. Furthermore, the BSID-II scores were evaluated and crosschecked by three separate examiners. Therefore, we believe that the rigorous care with which we used the BSID-II was sufficient to evaluate the effect of DLCs on neurodevelopment.

We were not able to examine influence of intellectual performance of the mothers on the BSID-II scores at 6 and 18 months, because the mothers' intellectual performance was only measured after their children were 18 months old (some mothers' intellectual performance was measured when their children were 42 months old). Our sample size, though relatively small to be considered representative of the general population, was similar or larger than those reported in several previous studies (Wilhelm et al., 2008b, Wilhelm et al., 2008a, Lynch et al., 2012). However, because the measurements of DLCs were highly complicated and cost too much, it was difficult to measure them in large number of the participants. There have been no studies, which have analyzed the association between the isomer levels of DLCs levels and a child's neurodevelopment. Therefore, we believed that analysis of the associations between DLCs and childrens' development in this study was essential. Additionally, the potential for selection bias remains as this cohort study was conducted at one hospital catering to residents of Sapporo and the surrounding areas. In this study, smoking rate during pregnancy was significantly lower in the 6-month-old (12.1%) and 18-month-old group (8.3%) than those reported for the entire Sapporo cohort mothers (17.1%). Therefore, we assume that the mothers participating in this child development survey may have relatively higher awareness of health problems in children. However, as our analysis was adjusted by socioeconomic status such as income, smoking, and child care environment, and as there were no significant differences in the DLC levels of the entire Sapporo cohort, the 6-month-old and 18-month-old group, we consider that our analysis and

interpretation of child neurodevelopment and DLCs levels were appropriate.

Taking into account the gestational anemia status of the mothers, approximately 25% of the maternal blood samples for both the 6-month-old and the 18-month-old groups were collected post-delivery. Accordingly, we adjusted the analyses for the time of blood sampling when examining the association between DLC levels and child development.

Lastly, there were approximately 70 subjects fewer in the 18-month-old group than in the 6-month-old group. Although the subject characteristics and DLC levels were similar in both age groups (Table 1 and 2), the smaller sample size may partly be responsible for the absence of adverse effects observed in this group. Additional follow-up studies are required to clarify the impact of each PCB isomer and dioxins on child neurodevelopment and their sex-specific differences.

5. Conclusion

In this study, we report sex-specific differences in the effect of PCB and dioxin exposure on child neurodevelopment. Furthermore, our results highlight the importance of examining sex hormone levels in relation to the postnatal behavioral development or sexual characteristics.

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Table 1. Characteristics of mothers and children in 6months and 18months

Characteristic	No. (%)	
	6months (n=190)	18months (n=121)
Maternal characteristics		
Age (years)	31.1 ± 4.7 [a]	31.4 ± 4.7 [a]
Educational level (years)		
≤12	71 (37.4)	48 (39.7)
≥13	119 (62.6)	73 (60.3)
Economic status: annual income (yen)		
<3,000,000	28 (14.7)	15 (12.4)
3,000,000–5,000,000	89 (46.8)	62 (51.2)
≥5,000,000	46 (24.2)	27 (22.3)
Working during pregnancy	53 (27.9)	39 (32.2)
Smoking during pregnancy	23 (12.1)	10 (8.3)
Fish intake during pregnancy		
Inshore fish		
≤2 time/month; rarely/never	108 (56.8)	70 (57.9)
>2 time/month; <3 time/week	71 (37.4)	42 (34.7)
≥3 time/week	11 (5.8)	9 (7.4)
Deep-sea fish		
≤2 time/month; rarely/never	89 (46.8)	53 (43.8)
>2 time/month; <3 time/week	94 (49.5)	63 (52.1)
≥3 time/week	7 (3.7)	5 (4.1)
Caffeine intake during pregnancy (mg/day)	137.1 ± 92.8 [a]	123.5 ± 71.6 [a]
Alcohol intake before pregnancy (g/day)	18.7 ± 63.5 [a]	13.7 ± 29.5 [a]
Alcohol intake during pregnancy (g/day)	1.0 ± 2.6 [a]	1.0 ± 2.7 [a]
Blood sampling time		
During pregnancy	143 (75.3)	90 (74.3)
After delivery	47 (24.7)	31 (25.6)
Child characteristics		
Sex		
Male	99 (52.1)	60 (49.6)
Female	91 (47.9)	61 (50.4)
Gestational age (days)	277.2 ± 8.1 [a]	276.2 ± 7.7 [a]
Birthweight (g)	3124.8 ± 332.1 [a]	3120.2 ± 314.8 [a]
Length (cm)	48.3 ± 1.5 [a]	48.4 ± 1.6 [a]
Head circumference (cm)	33.4 ± 1.3 [a]	33.4 ± 1.3 [a]
First-born	99 (52.1)	56 (46.3)
Duration of breastfeeding, ≥3months	109 (57.4)	-
Duration of breastfeeding (months)	-	10.9 ± 6.0 [a]
Age at testing (days)	187.0 ± 4.5 [a]	571.4 ± 7.5 [a]
BSID-II Mental Index Score; MDI	90.6 ± 5.8 [a]	82.2 ± 10.7 [a]
BSID-II Motor Index Score; PDI	89.9 ± 10.7 [a]	85.2 ± 10.9 [a]
Index of Child Care Environment	22.7 ± 2.6 [b]	28.0 ± 3.6 [b]

[a]mean ± SD. [b]30 points of perfect scores in 6 months and 38 points of perfect scores in 18 months.

Table2. Level of dioxin like PCB and PCDD/PCDF (pg/g lipid) in maternal blood in 18 months (N=121)

	Detection limit[a]	25th[b]	50th[b]	75th[b]
PCDD				
2,3,7,8-TCDD	1.0	ND	1.0	1.3
1,2,3,7,8-PeCDD	1.0	3.2	4.0	5.1
1,2,3,4,7,8-HxCDD	2.0	ND	ND	2.3
1,2,3,6,7,8-HxCDD	2.0	10.2	13.5	18.5
1,2,3,7,8,9-HxCDD	2.0	ND	2.3	3.0
1,2,3,4,6,7,8-HpCDD	2.0	19.0	24.3	31.6
OCDD	4.0	331.8	438.5	580.7
PCDF				
2,3,7,8-TCDF	1.0	ND	ND	1.1
1,2,3,7,8-PeCDF	1.0	ND	ND	ND
2,3,4,7,8-PeCDF	1.0	4.4	5.8	7.4
1,2,3,4,7,8-HxCDF	2.0	ND	2.5	3.2
1,2,3,6,7,8-HxCDF	2.0	2.1	2.7	3.6
2,3,4,6,7,8-HxCDF	2.0	ND	ND	ND
1,2,3,7,8,9-HxCDF	2.0	ND	ND	ND
1,2,3,4,6,7,8-HpCDF	2.0	ND	2.4	3.2
1,2,3,4,7,8,9-HpCDF	2.0	ND	ND	ND
OCDF	4.0	ND	ND	ND
non-ortho PCB				
33'4'4'-TCB(#77)	10.0	10.1	12.3	15.7
344'5-TCB(#81)	10.0	ND	ND	ND
33'44'5-PenCB(#126)	10.0	25.4	38.5	57.0
33'44'55'-HxCB(#169)	10.0	18.9	26.8	35.7
mono-ortho PCB				
233'44'-PenCB(#105)	10.0	1006.0	1507.0	2206.7
2344'5-PenCB(#114)	10.0	235.9	356.1	505.2
23'44'5-PenCB(#118)	10.0	4016.2	6266.2	8812.5
2'344'5-PenCB(#123)	10.0	72.8	119.8	164.5
233'44'5-HexCB(#156)	10.0	1359.0	1961.8	2713.0
233'44'5'-HexCB(#157)	10.0	324.3	502.4	685.0
23'44'55'-HexCB(#167)	10.0	489.4	799.2	1019.1
233'44'55'-HpCB(#189)	10.0	157.9	235.9	326.2
Total				
Total PCDD		367.1	488.5	642.8
Total PCDF		16.3	19.7	22.9
Total PCDD/PCDF		387.3	505.9	669.2
Total Non-ortho PCBs		61.3	83.9	110.3
Total Mono-ortho PCBs		7775.9	12115.8	15677.0
Total Coplanar PCB		7851.5	12203.5	15790.0
Total Dioxin		8300.9	12635.5	16557.8
WHO-05				
Total PCDD TEQ		5.4	7.2	9.4
Total PCDF TEQ		2.0	2.6	3.2

Total PCDD/PCDF TEQ	7.4	9.8	12.5
Total non-ortho PCB TEQ	3.1	4.7	6.6
Total mono-ortho PCB TEQ	0.2	0.4	0.5
Total coplanar PCB TEQ	3.3	5.1	7.1
Total Dioxin-TEQ	11.2	15.1	19.6

Abbreviations: ND = non-detectable,

[a]For subjects with a level below the detection limit, we inputted a value equal to half the detection limit.

[b]Percentiles.

Table3. Multiple linear regression for effects DLCs in maternal blood on MDI and PDI scores for 6- and 18-month-old male children

	6M MDI			6M PDI			18M MDI		18M PDI	
	β[a]	t		β[a]	t		β[a]	t	*β*[a]	*t*
PCDD										
2,3,7,8-TCDD	-0.16	-1.45		-0.24	-2.34	*	-0.09	-0.67	-0.16	-0.99
1,2,3,7,8-PeCDD	-0.06	-0.50		-0.11	-1.05		-0.08	-0.55	-0.05	-0.29
1,2,3,4,7,8-HxCDD	-0.16	-1.41		-0.20	-1.82		-0.02	-0.15	-0.06	-0.39
1,2,3,6,7,8-HxCDD	0.01	0.08		-0.06	-0.61		0.00	-0.04	0.05	0.30
1,2,3,7,8,9-HxCDD	-0.02	-0.18		-0.14	-1.32		0.00	-0.02	-0.01	-0.05
1,2,3,4,6,7,8-HpCDD	-0.27	-2.48	*	-0.27	-2.61	*	-0.03	-0.19	-0.03	-0.18
OCDD	-0.09	-0.85		-0.24	-2.36	*	0.01	0.11	-0.14	-0.99
PCDF										
2,3,7,8-TCDF	-0.08	-0.77		-0.23	-2.37	*	-0.05	-0.38	0.02	0.12
1,2,3,7,8-PeCDF	-0.02	-0.15		-0.22	-2.28	*	-0.10	-0.84	0.16	1.18
2,3,4,7,8-PeCDF	0.00	0.04		-0.16	-1.58		-0.15	-1.08	-0.10	-0.63
1,2,3,4,7,8-HxCDF	-0.05	-0.49		-0.17	-1.64		-0.07	-0.48	-0.12	-0.79
1,2,3,6,7,8-HxCDF	-0.03	-0.25		-0.15	-1.40		-0.04	-0.26	-0.11	-0.73
2,3,4,6,7,8-HxCDF	0.05	0.45		-0.17	-1.72		-0.04	-0.32	0.09	0.61
1,2,3,7,8,9-HxCDF	ND	ND		ND	ND		ND	ND	ND	ND
1,2,3,4,6,7,8-HpCDF	-0.06	-0.60		-0.04	-0.40		-0.09	-0.67	-0.18	-1.19
1,2,3,4,7,8,9-HpCDF	ND	ND		ND	ND		ND	ND	ND	ND
OCDF	-0.07	-0.64		-0.07	-0.75		-0.02	-0.15	-0.16	-1.13
non-ortho PCB										
33'4'4'-TCB(#77)	0.04	0.39		-0.11	-1.16		0.02	0.13	0.01	0.08
344'5-TCB(#81)	ND	ND		ND	ND		ND	ND	ND	ND
33'44'5-PenCB(#126)	-0.03	-0.24		-0.19	-1.86		-0.16	-1.24	0.00	-0.02
33'44'55'-HxCB(#169)	-0.03	-0.23		-0.19	-1.77		-0.11	-0.81	-0.06	-0.38
mono-ortho PCB										
233'44'-PenCB(#105)	-0.02	-0.17		-0.18	-1.83		-0.05	-0.41	0.04	0.29
2344'5-PenCB(#114)	-0.06	-0.60		-0.21	-2.08	*	-0.06	-0.44	-0.03	-0.23
23'44'5-PenCB(#118)	-0.05	-0.46		-0.19	-1.96		-0.09	-0.73	0.04	0.32
2'344'5-PenCB(#123)	0.03	0.31		-0.16	-1.60		-0.11	-0.83	0.06	0.43
233'44'5-HexCB(#156)	-0.08	-0.70		-0.23	-2.29	*	-0.12	-0.86	-0.03	-0.18
233'44'5'-HexCB(#157)	-0.09	-0.85		-0.24	-2.36	*	-0.11	-0.84	-0.02	-0.14
23'44'55'-HexCB(#167)	-0.05	-0.46		-0.24	-2.51	*	-0.11	-0.89	0.00	0.02
233'44'55'-HpCB(#189)	-0.17	-1.64		-0.22	-2.28	*	-0.12	-0.91	0.00	0.00
Total										
Total PCDD	-0.10	-0.91		-0.24	-2.36	*	0.01	0.12	-0.13	-0.89
Total PCDF	-0.05	-0.41		-0.18	-1.77		-0.13	-0.93	-0.15	-0.99
Total PCDD/PCDF	-0.10	-0.91		-0.24	-2.37	*	0.01	0.08	-0.13	-0.90
Total Non-ortho PCBs	0.00	0.01		-0.20	-1.96		-0.13	-0.98	-0.02	-0.12
Total Mono-ortho PCBs	-0.05	-0.48		-0.21	-2.16	*	-0.10	-0.79	0.03	0.18
Total Coplanar PCB	-0.05	-0.48		-0.21	-2.16	*	-0.10	-0.79	0.03	0.17
Total Dioxin	-0.05	-0.49		-0.22	-2.23	*	-0.10	-0.79	0.03	0.18
WHO-05										
Total PCDD TEQ	-0.07	-0.65		-0.15	-1.36		-0.06	-0.42	-0.04	-0.24

Total PCDF TEQ	-0.01	-0.06	-0.19	-1.80		-0.14	-1.02	-0.10	-0.68
Total PCDD/PCDF TEQ	-0.06	-0.53	-0.16	-1.51		-0.08	-0.58	-0.06	-0.35
Total non-ortho PCB TEQ	-0.02	-0.18	-0.19	-1.91		-0.15	-1.21	-0.01	-0.07
Total mono-ortho PCB TEQ	-0.05	-0.48	-0.21	-2.16	*	-0.10	-0.79	0.03	0.18
Total coplanar PCB TEQ	-0.05	-0.48	-0.21	-2.16	*	-0.10	-0.79	0.03	0.17
Total Dioxin-TEQ	-0.04	-0.34	-0.18	-1.72		-0.12	-0.90	-0.01	-0.08

Abbreviations: ND = non-detectable; WHO = World Health Organization.

[a]β is the point increase in developmental score per PCB and dioxin level (common logarithm) adjusted for gestational age (days), caffeine intake during pregnancy (mg/day), economic status; an annual income, index of child care Environment, smoking during pregnancy, blood sampling time and fish intake during pregnancy (Inshore fish and Deep-sea fish). *$p<0.05$, **$p<0.01$.

Table4. Multiple linear regression for effects DLCs in maternal blood on MDI and PDI scores for 6- and 18-month-old female children

	6M MDI			6M PDI			18M MDI			18M PDI	
	β[a]	t		β[a]	t		β[a]	t		*β*[a]	*t*
PCDD											
2,3,7,8-TCDD	-0.03	-0.30		-0.01	-0.08		0.15	1.04		-0.17	-1.19
1,2,3,7,8-PeCDD	0.26	2.37	*	0.01	0.07		0.19	1.41		-0.18	-1.24
1,2,3,4,7,8-HxCDD	0.08	0.76		-0.04	-0.38		0.04	0.32		-0.19	-1.33
1,2,3,6,7,8-HxCDD	0.13	1.17		-0.09	-0.84		0.25	1.82		-0.20	-1.39
1,2,3,7,8,9-HxCDD	0.12	1.05		-0.20	-1.84		0.07	0.47		-0.28	-1.84
1,2,3,4,6,7,8-HpCDD	-0.10	-0.92		-0.13	-1.19		0.11	0.78		-0.08	-0.55
OCDD	-0.14	-1.28		-0.13	-1.18		0.19	1.32		0.02	0.14
PCDF											
2,3,7,8-TCDF	-0.09	-0.85		-0.11	-1.03		-0.03	-0.24		0.07	0.53
1,2,3,7,8-PeCDF	-0.06	-0.51		-0.16	-1.51		0.12	0.91		-0.05	-0.37
2,3,4,7,8-PeCDF	0.18	1.52		-0.06	-0.54		0.25	1.71		-0.10	-0.64
1,2,3,4,7,8-HxCDF	-0.05	-0.38		-0.17	-1.45		0.02	0.11		-0.24	-1.60
1,2,3,6,7,8-HxCDF	0.10	0.86		-0.08	-0.66		0.24	1.45		-0.20	-1.14
2,3,4,6,7,8-HxCDF	0.09	0.81		-0.12	-1.17		-0.12	-0.94		0.01	0.09
1,2,3,7,8,9-HxCDF											
1,2,3,4,6,7,8-HpCDF	0.04	0.37		-0.07	-0.62		0.34	2.42	*	0.02	0.11
1,2,3,4,7,8,9-HpCDF	ND	ND		ND	ND		ND	ND		ND	ND
OCDF	ND	ND		ND	ND		ND	ND		ND	ND
non-ortho PCB											
33'4'4'-TCB(#77)	0.06	0.52		-0.06	-0.60		0.14	0.94		0.15	1.03
344'5-TCB(#81)	ND	ND		ND	ND		-0.11	-0.86		-0.07	-0.55
33'44'5-PenCB(#126)	0.00	0.04		-0.21	-1.93		0.03	0.18		0.02	0.10
33'44'55'-HxCB(#169)	0.10	0.89		-0.06	-0.57		0.33	2.29	*	0.00	0.00
mono-ortho PCB											
233'44'-PenCB(#105)	0.02	0.18		-0.16	-1.50		0.14	1.06		0.08	0.55
2344'5-PenCB(#114)	0.09	0.81		-0.14	-1.31		0.34	2.54	*	0.05	0.33
23'44'5-PenCB(#118)	0.03	0.31		-0.18	-1.67		0.18	1.34		0.04	0.29
2'344'5-PenCB(#123)	0.03	0.23		-0.22	-2.08	*	0.11	0.77		0.06	0.39
233'44'5-HexCB(#156)	0.11	0.95		-0.09	-0.80		0.29	2.20	*	-0.03	-0.21
233'44'5'-HexCB(#157)	0.11	0.96		-0.15	-1.41		0.34	2.65	*	-0.03	-0.24
23'44'55'-HexCB(#167)	0.03	0.28		-0.09	-0.85		0.26	1.95		-0.02	-0.15
233'44'55'-HpCB(#189)	-0.04	-0.32		-0.12	-1.16		0.31	2.40	*	0.06	0.43
Total											
Total PCDD	-0.13	-1.18		-0.13	-1.19		0.20	1.34		0.00	0.02
Total PCDF	0.08	0.69		-0.12	-0.99		0.24	1.55		-0.10	-0.59
Total PCDD/PCDF	-0.13	-1.14		-0.13	-1.20		0.20	1.36		0.00	-0.01
Total Non-ortho PCBs	0.04	0.35		-0.14	-1.29		0.15	1.02		0.03	0.22
Total Mono-ortho PCBs	0.05	0.44		-0.16	-1.50		0.23	1.67		0.03	0.19
Total Coplanar PCB	0.05	0.44		-0.16	-1.50		0.22	1.67		0.03	0.19
Total Dioxin	0.04	0.38		-0.16	-1.53		0.23	1.69		0.02	0.16
WHO-05											
Total PCDD TEQ	0.17	1.50		-0.04	-0.36		0.22	1.55		-0.23	-1.59

Total PCDF TEQ	0.13	1.14	-0.08	-0.70	0.24	1.57	-0.10	-0.66
Total PCDD/PCDF TEQ	0.16	1.44	-0.05	-0.46	0.23	1.57	-0.20	-1.36
Total non-ortho PCB TEQ	0.03	0.23	-0.18	-1.68	0.07	0.51	0.02	0.10
Total mono-ortho PCB TEQ	0.05	0.44	-0.16	-1.50	0.23	1.67	0.03	0.19
Total coplanar PCB TEQ	0.05	0.44	-0.16	-1.50	0.22	1.67	0.03	0.19
Total Dioxin-TEQ	0.12	1.05	-0.10	-0.95	0.18	1.22	-0.13	-0.83

Abbreviations: ND = non-detectable; WHO = World Health Organization.

[a]β is the point increase in developmental score per PCB and dioxin level (common logarithm) adjusted for gestational age (days), caffeine intake during pregnancy (mg/day), economic status; an annual income, index of child care Environment, smoking during pregnancy, blood sampling time and fish intake during pregnancy (Inshore fish and Deep-sea fish). *$p<0.05$, **$p<0.01$.

Table A1. BSID-II mental (MDI) and psychomotor (PDI) development scores for infants at 6months in relation to mothers and children characteristic

Characteristic	male (n=99)				female (n=91)			
		MDI		PDI		MDI		PDI
	No.	Mean ± SD		Mean ± SD	No.	Mean ± SD		Mean ± SD
Maternal characteristics								
Age (years)		r = –0.16		r = –0.16		r = 0.15		r = 0.03
Educational level (years)								
≤12	42	91.3 ± 4.2		92.1 ± 10.7	29	91.4 ± 4.6		88.9 ± 10.0
≥13	57	90.6 ± 6.8		88.8 ± 11.0	62	89.7 ± 6.1		90.0 ± 10.7
Economic status: annual income (yen)								
<3,000,000	19	90.3 ± 6.5		87.8 ± 9.9	9	90.9 ± 3.8		89.0 ± 9.1
3,000,000–5,000,000	44	91.2 ± 5.3		90.5 ± 11.2	45	90.0 ± 6.4		91.5 ± 11.0
≥5,000,000	36	90.8 ± 6.2		90.2 ± 10.9	37	90.4 ± 5.3		87.6 ± 9.9
Working during pregnancy								
No	87	90.3 ± 5.5	**	89.8 ± 10.7	81	90.3 ± 5.8		90.1 ± 10.5
Yes	12	95.3 ± 6.6		93.0 ± 12.9	10	89.6 ± 4.9		85.9 ± 8.8
Smoking during pregnancy								
No	89	90.8 ± 5.7		90.5 ± 11.2	78	90.5 ± 5.8		90.0 ± 10.5
Yes	10	91.7 ± 7.1		87.5 ± 8.6	13	88.5 ± 5.4		88.3 ± 10.0
Fish intake during pregnancy								
Inshore fish								
≤2 time/month; rarely/never	57	90.7 ± 6.1		90.3 ± 11.7	52	90.4 ± 5.3		89.5 ± 10.8
>2 time/month; <3 time/week	37	91.5 ± 5.3		90.2 ± 10.5	34	89.8 ± 6.5		90.2 ± 10.5
≥3 time/week	6	87.3 ± 5.8		88.5 ± 5.8	5	91.2 ± 5.0		86.8 ± 5.0
Deep-sea fish								
≤2 time/month; rarely/never	45	91.5± 5.4		91.4 ± 10.3	44	90.3± 5.5		89.5 ± 10.2
>2 time/month; <3 time/week	50	90.0 ± 6.2		89.2 ± 11.6	44	90.2 ± 6.1		90.3 ± 10.2
≥3 time/week	4	95.0 ± 1.2		88.8 ± 10.2	3	90.0 ± 6.0		82.0 ± 16.7
Caffeine intake during pregnancy (mg/day)		r = –0.00		r = –0.16		r = –0.08		r = –0.21 *
Alcohol intake before pregnancy (g/day)		r = 0.07		r = –0.08		r = –0.16		r = 0.05
Alcohol intake during pregnancy (g/day)		r = 0.01		r = –0.09		r = –0.09		r = 0.05
Blood sampling time								

During pregnancy	75	91.1 ± 5.5		89.9 ± 10.9		75	91.1 ± 5.5	89.9 ± 10.9
After delivery	24	90.3 ± 6.9		91.1 ± 11.3		24	90.3 ± 6.9	91.1 ± 11.3
Child characteristics								
Gestational age (days)		r = 0.23	*	r =0.27	**		r = 0.06	r =0.01
First-born								
Yes	46	92.4 ± 5.4	*	92.0 ± 13.0		53	90.3 ± 5.1	90.2 ± 9.3
No	53	89.6 ± 5.9		88.7 ± 8.6		38	90.1 ± 6.6	88.8 ± 11.9
Duration of breastfeeding, ≥3months								
Yes	55	91.1 ± 6.4		90.8 ± 11.3		54	90.3 ± 6.3	89.8 ± 10.0
No	54	90.7 ± 5.2		89.5 ± 10.5		37	90.1 ± 5.0	89.4 ± 11.1
Index of Child Care Environment		r = 0.06		r = 0.01			r = –0.06	r = –0.19

Student's t-test, One-way analysis of variance, Spearman's correlation coefficient test. * $p < 0.05$; ** $p < 0.01$.

Table A2. BSID-II mental (MDI) and psychomotor (PDI) development scores for children at 18months in relation to mothers and children characteristic

Characteristic	male (n=60)				female (n=61)			
		MDI		PDI		MDI		PDI
	No.	Mean ± SD		Mean ± SD	No.	Mean ± SD		Mean ± SD
Maternal characteristics								
Age (years)		r = –0.01		r = 0.09		r = 0.19		r = –0.04
Educational level (years)								
≤12	29	81.3 ± 11.1		85.9 ± 14.2	19	84.6 ± 12.0		85.4 ± 7.8
≥13	31	83.4 ± 11.4		86.3± 9.5	42	80.6 ± 9.2		84.0± 10.6
Economic status: annual income (yen)								
<3,000,000	9	78.1± 6.9		83.8 ± 14.6	6	80.3± 6.2	*	85.8 ± 9.5
3,000,000–5,000,000	29	81.1 ± 12.2		85.9 ± 12.3	33	79.3 ± 10.0		83.1 ± 9.0
≥5,000,000	22	86.5 ± 10.6		87.1 ± 10.5	22	86.2 ± 10.3		86.1 ± 11.1
Working during pregnancy								
No	51	81.6 ± 11.1		86.5 ± 12.5	56	81.8 ± 10.5		85.0 ± 9.8
Yes	9	88.6 ± 10.7		83.3 ± 7.5	5	82.2 ± 6.3		78.2 ± 8.7
Smoking during pregnancy								
No	56	83.4 ± 10.9	*	86.5± 11.8	55	81.9 ± 10.3		84.1± 9.9
Yes	4	71.5 ± 11.6		80.0 ± 12.4	6	81.8 ± 10.1		87.3 ± 9.4
Fish intake during pregnancy								
Inshore fish								
≤2 time/month; rarely/never	35	82.6 ±9.7		87.5 ± 11.0	35	80.6 ±10.4		84.9 ± 9.9
>2 time/month; <3 time/week	20	82.3 ± 14.1		84.1 ± 13.2	22	83.3 ± 10.4		84.7 ± 10.0
≥3 time/week	5	83.8 ±11.2		83.6 ± 13.4	4	85.5 ±6.8		79.0 ± 8.7
Deep-sea fish								
≤2 time/month; rarely/never	25	84.2 ± 11.7		87.3 ± 10.8	28	79.4 ± 8.9		82.8 ± 7.9
>2 time/month; <3 time/week	31	81.3 ± 11.6		85.1 ± 13.4	32	84.0 ± 11.1		86.2 ± 11.0
≥3 time/week	4	83.0 ± 2.8		85.0 ± 3.8	1	83		74
Caffeine intake during pregnancy (mg/day)		r = –0.11		r = –0.09		r = –0.070		r = –0.00
Alcohol intake before pregnancy (g/day)		r = –0.22		r = –0.22		r = –0.10		r = 0.15
Alcohol intake during pregnancy (g/day)		r = 0.01		r = –0.08		r = –0.04		r = 0.26 *
Blood sampling time								

During pregnancy	45	82.7 ± 11.1		85.7± 12.3	45	82.1 ± 10.3	84.4± 8.7	
After delivery	15	82.2 ± 12.1		87.1 ± 11.0	16	81.2 ± 10.1	84.5 ± 12.7	
Child characteristics								
Gestational age (days)		r = 0.21		r = 0.09		r = 0.11	r = 0.22	
First-born								
Yes	26	81.5 ± 11.6		85.7 ± 12.6	30	80.3 ± 9.2	82.2 ± 7.6	
No	34	83.5 ± 11.1		86.3 ± 11.5	31	83.4 ± 11.1	86.6 ± 11.3	
Duration of breastfeeding (months)		r = 0.05		r = 0.22		r = –0.09	r = –0.27	*
Index of Child Care Environment		r = 0.33	*	r = 0.21		r = 0.14	r = –0.00	

Student's t-test, One-way analysis of variance, Spearman's correlation coefficient test. * $p < 0.05$; ** $p < 0.01$.